Title: CHARACTERIZATION OF THE RELEASE AND TRANSFER OF ENDOTHELIUM-DERIVED RELAXING FACTOR (EDRF) FROM PULMONARY ARTERY ENDOTHELIUM

Authors: R. A. Johns, M.D.*, N. J. Izzo, B.S.**, M. J. Peach, Ph.D.**

Affiliation: Departments of *Anesthesiology and **Pharmacology, University of Virginia, Charlottesville, VA 22908

Introduction: Responses of the pulmonary circulation are important to the anesthesiologist in both physiologic (hypoxic pulmonary vasoconstriction) and pathophysiologic (pulmonary hypertension, ARDS) situations. The role of the endothelium in vascular reactivity has taken on increasing interest since the description of EDRF in 1980.[1] We have 1) investigated the ability of several agonists to release EDRF from rabbit pulmonary artery rings and from cultured bovine pulmonary endothelial cells, 2) demonstrated a concomitant rise in endothelial cytosolic calcium concentration, and 3) an accompanying rise in vascular smooth muscle soluble cyclic GMP.

Methods: Rabbit main pulmonary artery rings with or without endothelium were hung in water-jacketed baths of 37°C Krebs solution containing 28mM indomethacin (to block any component of the relaxation due to prostacyclin), and tension was measured using Grass FT-03 transducers. Optimal resting tension was determined by preliminary length-tension curves, and active tension was applied using an EC60 dose of phenylephrine (10^{-6} M). Relaxation-response curves to methacholine (10^{-8} to 10^{-5} M), melittin (1, 2, and 3 µg/ml), arachidonic acid (3, 10, 30, and 100 µM), and the calcium ionophore A23187 (10^{-9} to 10^{-6} M) were determined.

For determination of cyclic GMP responses to EDRF, isolated bovine pulmonary artery endothelial and rat vascular smooth muscle cells were grown in mixed culture and stimulated with EDRF agonists at concentrations which provided maximal relaxation in vascular rings. Cyclic GMP was extracted with 0.1 N HCl and cytosolic concentrations determined by radioimmunoassay.

The transfer of EDRF from cultured endothelial cells also was bioassayed by growing the cells on Cytodex 3 microcarrier beads, packing them loosely in a column, suffusing the column with Krebs solution with or without EDRF agonists and observing the ability of the effluent to relax endothelium-denuded rabbit aorta rings.

For intracellular calcium measurements, cultured pulmonary endothelial cell suspensions were incubated with Fura-2/AM (an agent which fluoresces when it binds calcium) for 30 min, washed twice, and resuspended (3×10^5 cells/ml) in a balanced salt solution containing 1.0 mM $CaCl_2$. The cells were stimulated with EDRF agonists, and fluorescence was measured using a SPEX Industries fluorometer with samples kept at 37° C and stirred continuously.

Data were analyzed by student's t-tests or by ANOVA with Dunnett's test.

Results: All of the EDRF agonists relaxed endothelium-intact rings in a dose-related manner but had no effect on endothelium-denuded vessels. Figure 1 presents the data for the maximum relaxation response in endothelium-intact rings (n=5-7, $p<0.01$). In mixed culture experiments, melittin 3µg/ml, arachidonate 100µM, and calcium ionophore 10^{-7}M produced significant ($p<0.01$, n=4) increases in cGMP of 9.27, 2.25, and 6.26 times control, respectively. Figure 2 demonstrates the relaxation response of a denuded rabbit aortic ring to EDRF released and transferred from cultured pulmonary artery endothelial cells stimulated with bradykinin (BK), melittin (mel), and arachidonic acid (AA). Stimulation of endothelial cells loaded with Fura-2 by bradykinin, melittin, or calcium ionophore resulted in increased flourescence consistent with increases in cytosolic calcium.

Discussion: We have demonstrated endothelium-dependent relaxation in isolated rabbit pulmonary arteries as well as the release and transfer of EDRF from cultured pulmonary artery endothelial cells. The formation or release of EDRF from endothelial cells is accompanied by increased cytosolic calcium, and its action on vascular smooth muscle correlates with increases in cGMP concentrations. The potential role of EDRF in the physiology and pathophysiology of the pulmonary circulation including hypoxic pulmonary vasoconstriction, pulmonary hypertension, and ARDS is under investigation.

References.

1. Furchgott R, Zawadski D: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288:373-376, 1980

Figure 1

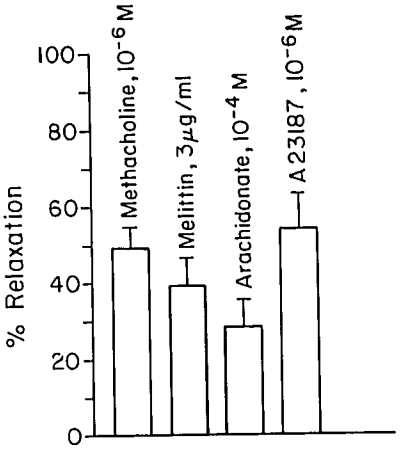

Figure 2